

1. A non-naturally occurring nucleic acid comprising:

(A) a first nucleotide sequence encoding at least one AAV Rep protein; and

(B) a second nucleotide sequence encoding at least one AAV Cap protein,

wherein the second nucleotide sequence comprises (i) a polynucleotide encoding a portion of a  
5 Cap protein found in an AAV of a first serotype but not in an AAV of a second serotype  
differing from the first serotype and (ii) a polynucleotide encoding a portion of a Cap protein  
found in the AAV of the second serotype, but not in the AAV of the first serotype.

2. The non-naturally occurring nucleic acid of claim 1, wherein the nucleic acid  
10 further comprises a first AAV TR and a second AAV TR, and the first and second nucleotide  
sequences are interposed between the first and the second AAV TRs.

3. The non-naturally occurring nucleic acid of claim 2, wherein the first and the  
second AAV TRs are from serotype 2.

4. The non-naturally occurring nucleic acid of claim 1, wherein the first serotype is  
serotype 1.

5. The non-naturally occurring nucleic acid of claim 1, wherein the first serotype is  
20 serotype 2.

6. The non-naturally occurring nucleic acid of claim 1, wherein the first serotype is  
serotype 3.

7. The non-naturally occurring nucleic acid of claim 1, wherein the first serotype is  
25 serotype 4.

8. The non-naturally occurring nucleic acid of claim 1, wherein the first serotype is  
serotype 5.

9. The non-naturally occurring nucleic acid of claim 1, wherein the first serotype is  
30 serotype 6.

10. The non-naturally occurring nucleic acid of claim 1, wherein the first serotype is serotype 7.

11. The non-naturally occurring nucleic acid of claim 1, wherein the first serotype is serotype 8.

12. The non-naturally occurring nucleic acid of claim 1, wherein the nucleic acid is comprised within a vector.

13. The non-naturally occurring nucleic acid of claim 1, wherein the AAV Rep protein is from serotype 2.

14. The non-naturally occurring nucleic acid of claim 1, wherein the nucleic acid further comprises a third nucleotide sequence encoding at least one molecule providing helper function.

15. The non-naturally occurring nucleic acid of claim 14, wherein the third nucleotide sequence encoding at least one molecule providing helper function is a polynucleotide from a virus selected from the group consisting of: adenovirus and herpesvirus.

16. A vector library comprising at least a first vector and a second vector, the first vector comprising a nucleic acid comprising:

(A) a first nucleotide sequence encoding at least one AAV Rep protein; and

(B) a second nucleotide sequence encoding at least one AAV Cap protein,

wherein the second nucleotide sequence comprises (i) a polynucleotide encoding a portion of a Cap protein found in an AAV of a first serotype but not in an AAV of a second serotype differing from the first serotype, and (ii) a polynucleotide encoding a portion of a Cap protein found in the AAV of the second serotype, but not in the AAV of the first serotype, and the second vector differing from the first vector by at least one nucleotide.

17. The vector library of claim 16, wherein the vector library is incorporated into at least one host cell.

18. The vector library of claim 17, wherein the host cell is an insect cell.

19. An AAV virion comprising a nucleic acid comprising:

(A) a first nucleotide sequence encoding at least one AAV Rep protein;

(B) a second nucleotide sequence encoding at least one AAV Cap protein,

wherein the second nucleotide sequence comprises (i) a polynucleotide encoding a portion of a Cap protein found in an AAV of a first serotype but not in an AAV of a second serotype differing from the first serotype, and (ii) a polynucleotide encoding a portion of a Cap protein found in the AAV of the second serotype, but not in the AAV of the first serotype; and

(C) a first and a second AAV TR, wherein the first and the second nucleotide sequences are interposed between the first and the second AAV TRs.

20. The AAV virion of claim 19, wherein the first and second TRs are from serotype

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21. The AAV virion of claim 19, wherein the virion comprises an AAV Cap protein.

22. The AAV virion of claim 21, wherein the Cap protein is a WT Cap protein.

23. The AAV virion of claim 21, wherein the second nucleotide sequence further comprises a polynucleotide encoding a portion of a Cap protein found in an AAV of a third serotype but not found in a Cap protein of an AAV of the first or second serotypes.

24. The AAV virion of claim 23, wherein the second nucleotide sequence further comprises a polynucleotide encoding a portion of a Cap protein found in an AAV of a fourth serotype but not found in a Cap protein of an AAV of the first, second or third serotypes.

25. The AAV virion of claim 24, wherein the second nucleotide sequence further comprises a polynucleotide encoding a portion of a Cap protein found in an AAV of a fifth serotype but not found in a Cap protein of an AAV of the first, second, third or fourth serotypes.

26. The AAV virion of claim 25, wherein the second nucleotide sequence further comprises a polynucleotide encoding a portion of a Cap protein found in an AAV of a sixth

serotype but not found in a Cap protein of an AAV of the first, second, third, fourth or fifth serotypes.

27. The AAV virion of claim 26, wherein the second nucleotide sequence further comprises a polynucleotide encoding a portion of a Cap protein found in an AAV of a seventh serotype but not found in a Cap protein of an AAV of the first, second, third, fourth, fifth or sixth serotypes.

28. The AAV virion of claim 27, wherein the second nucleotide sequence further comprises a polynucleotide encoding a portion of a Cap protein found in an AAV of an eighth serotype but not found in a Cap protein of an AAV of the first, second, third, fourth, fifth, sixth or seventh serotypes.

29. The AAV virion of claim 22, wherein the WT AAV Cap protein is from serotype 2.

30. The AAV virion of claim 19, wherein the AAV Rep protein is from serotype 2.

31. The AAV virion of claim 19, wherein the AAV virion is incorporated into a host cell.

32. The AAV virion of claim 31, wherein the host cell is a mammalian cell.

33. The AAV virion of claim 19, further comprising at least one AAV Cap protein encoded by the second nucleotide sequence.

34. The AAV virion of claim 33, wherein the TRs are from AAV serotype 2.

35. An AAV virion comprising:

(A) a nucleic acid comprising:

(i) a first AAV TR;

(ii) a second AAV TR;

(iii) a non-AAV nucleic acid interposed between the first AAV TR

and the second AAV TR; and

(B) at least one AAV Cap protein, wherein the Cap protein is encoded by a nucleotide sequence comprising nucleic acid sequences from AAVs of at least a first serotype and a second serotype differing from the first serotype.

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36. The AAV virion of claim 35, wherein at least one of the TRs is from AAV serotype 2.

10 37. The AAV virion of claim 35, wherein the nucleotide sequence further comprises a nucleic acid sequence from an AAV of a third serotype differing from the first and second serotypes.

15 38. The AAV virion of claim 37, wherein the nucleotide sequence further comprises a nucleic acid sequence from an AAV of a fourth serotype differing from the first, second, and third serotypes.

20 39. The AAV virion of claim 38, wherein the nucleotide sequence further comprises a nucleic acid sequence from an AAV of a fifth serotype differing from the first, second, third and fourth serotypes.

40. The AAV virion of claim 39, wherein the nucleotide sequence further comprises a nucleic acid sequence from an AAV of a sixth serotype differing from the first, second, third, fourth and fifth serotypes.

25 41. The AAV virion of claim 40, wherein the nucleotide sequence further comprises a nucleic acid sequence from an AAV of a seventh serotype differing from the first, second, third, fourth, fifth, and sixth serotypes.

30 42. The AAV virion of claim 41, wherein the nucleotide sequence further comprises a nucleic acid sequence from an AAV of a eighth serotype differing from the first, second, third, fourth, fifth, sixth, and seventh serotypes.

43. The AAV virion of claim 35, wherein the nucleic acid further comprises an expression control sequence.

44. The AAV virion of claim 43, wherein the expression control sequence effects tissue-specific expression of the non-AAV nucleic acid.

45. The AAV virion of claim 43, wherein the expression control sequence comprises a promoter operably linked to the non-AAV nucleic acid.

46. The AAV virion of claim 35, wherein the non-AAV sequence encodes a therapeutic molecule.

47. The AAV virion of claim 46, wherein the therapeutic molecule is selected from the group consisting of: a polypeptide and a RNA.

48. The AAV virion of claim 35, wherein the virion is incorporated into a host cell.

49. The AAV virion of claim 48, wherein the host cell is a mammalian cell.

50. The AAV virion of claim 49, wherein the mammalian cell is a human cell.

51. A virion library comprising at least a first AAV virion and a second AAV virion, the first AAV virion comprising a nucleic acid comprising:

(A) a first nucleotide sequence encoding at least one AAV Rep protein;

(B) a second nucleotide sequence encoding at least one AAV Cap protein,

wherein the second nucleotide sequence comprises (i) a polynucleotide encoding a portion of a Cap protein found in an AAV of a first serotype but not in an AAV of a second serotype differing from the first serotype, and (ii) a polynucleotide encoding a portion of a Cap protein found in the AAV of the second serotype, but not in the AAV of the first serotype; and

(C) a first and a second AAV TR, wherein the first and the second nucleotide

sequences are interposed between the first and the second AAV TRs, and

the second AAV virion comprising a nucleic acid not comprised within the first AAV virion.

52. The virion library of claim 51, wherein the TRs are from serotype 2.

53. The virion library of claim 51, wherein the first and the second virions both  
5 comprise at least one WT AAV Cap protein.

54. The virion library of claim 51, wherein the virions are incorporated into at least  
one host cell.

10 55. The virion library of claim 54, wherein the host cell is an insect cell.

56. The virion library of claim 54, wherein the host cell is a mammalian cell.

57. The virion library of claim 51, the first and second virions further comprising at  
15 least one AAV Cap protein encoded by the second nucleotide sequence, wherein the second  
AAV virion comprises a Cap protein not comprised within the first AAV virion.

58. A method comprising the steps of:

(A) providing a first polynucleotide and a second polynucleotide, the first and  
20 second polynucleotides comprising an identical nucleotide sequence encoding at least a portion  
of an AAV Cap protein;

(B) mutating the first and second polynucleotides to generate a plurality of  
mutants, wherein each mutant comprises a nucleotide sequence encoding a portion of a Cap  
protein found in an AAV of a first serotype but not in an AAV of a second serotype differing  
25 from the first serotype;

(C) placing each of the plurality of mutants into vectors to form a plurality of  
vectors.

59. The method of claim 58, wherein the first serotype is serotype 1.

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60. The method of claim 58, wherein the first serotype is serotype 2.

61. The method of claim 58, wherein the first serotype is serotype 3.

62. The method of claim 58, wherein the first serotype is serotype 4.
63. The method of claim 58, wherein the first serotype is serotype 5.
- 5 64. The method of claim 58, wherein the first serotype is serotype 6.
65. The method of claim 58, wherein the first serotype is serotype 7.
- 10 66. The method of claim 58, wherein the first serotype is serotype 8.
67. The method of claim 58, wherein at least one of the vectors further comprises a nucleotide sequence encoding at least one AAV Rep protein.
- 15 68. The method of claim 67, wherein the Rep protein is from serotype 2.
69. The method of claim 58, further comprising the steps of:
- (D) introducing at least one of the vectors into a first host cell;
- (E) providing *rep* and *cap* gene products having WT functional activity to the
- 20 first host cell;
- (F) culturing the first host cell under conditions that allow production of a first population of virions containing the vectors, wherein the virions comprise at least one WT AAV Cap protein; and
- (F) harvesting the first population of virions from the first host cell.
- 25 70. The method of claim 69, wherein the first host cell is an insect cell.
71. The method of claim 69, wherein the *rep* and *cap* gene products are encoded by nucleic acids comprised within recombinant baculoviruses.
- 30 72. The method of claim 69, further comprising the steps of:



(G) infecting a second host cell with the first population of virions under conditions that allow production of a second population of virions, wherein each virion of the second population of virions comprises:

- (i) a first nucleotide sequence encoding at least one AAV Rep protein;
  - (ii) a second nucleotide sequence encoding at least one AAV Cap protein, wherein the nucleotide sequence comprises nucleic acid sequences from at least the first AAV serotype and the second AAV serotype; and
  - (iii) at least one AAV Cap protein encoded by the second nucleotide sequence; and
- (H) harvesting the second population of virions from the second host cell.

73. The method of claim 72, wherein the second host cell is a mammalian cell.

74. The method of claim 72, further comprising the steps of:

- (I) introducing the second population of virions into a target cell;
- (J) culturing the target cell under conditions that allow production of a third population of virions; and
- (K) harvesting the third population of virions from the target cell.

75. The method of claim 74, wherein the target cell is a mammalian cell.

76. The method of claim 75, wherein the mammalian cell is a human cell.

77. The method of claim 74, further comprising the steps of:

- (L) isolating a nucleic acid from the third population of virions that encodes an AAV Cap protein; and
- (M) determining the sequence of the nucleic acid isolated from the third population of virions.

78. The method of claim 77, further comprising the steps of:

- (N) placing the nucleic acid isolated from the third population of virions into a second vector;

- (O) introducing the second vector into a third host cell;
- (P) introducing into the third host cell a nucleotide sequence encoding at least one AAV Rep protein;
- 5 (Q) providing to the third host cell at least one molecule having helper function;
- (R) introducing into the third host cell a nucleic acid comprising:
- (i) a first AAV TR;
  - (ii) a second AAV TR; and
  - 10 (iii) a non-AAV nucleic acid interposed between the first AAV TR and the second AAV TR;
- (S) culturing the third host cell under conditions that allow production of virions, wherein the virions contain the nucleic acid of step (R); and
- (T) harvesting virions from the cell.

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